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A STUDY OF PNEUMOCOCCI FROM CASES OF INFECTIOUS ENDOCARDITIS.*†

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Certain cases of endocarditis with prolonged course reported by Schottmüller[1] and Horder[2] are similar clinically to those studied by Dr. F. Billings[3] and myself,[4] and the descriptions of the organisms isolated are very similar. Schottmüller designates his organisms as "Streptococcus mitior viridans," because they form long chains, are small in size, and produce green on blood-agar. He did not use inulin for differential tests, nor did he study the effects of animal passage. Horder names his organisms "saprophytic streptococci" on account of their lack of virulence and to distinguish them from Streptococcus pyogenes. The organisms studied by me appeared to be modified pneumococci, at least in most cases, and I now wish to present some of the results of a rather prolonged study of similar cocci from other cases of chronic infectious endocarditis.

FERMENTATIVE AND OTHER CHARACTERISTICS OF THE PNEUMOCOCCI IN ENDOCARDITIS.

Fluctuations in the fermentative powers of the cocci from endocarditis, as well as of the pneumococci isolated from pneumonia, have been observed repeatedly. Pneumococci from the sputum in pneumonia ferment inulin more strongly than those isolated from the blood, while pneumococci from cases of endocarditis ferment this carbohydrate still less and sometimes not at all. The following observations will illustrate this point: Repeated tests of the fermentative properties of the strain isolated on the many occasions in Case 362 (this Journal, p. 429) show that approximately only one-half of the colonies obtained from the blood on blood-agar plates fermented inulin. Similar results were obtained in the other cases studied.

The strain isolated July 7 from Case 408 (p. 431) fermented

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[1] *Münch. med. Wchnschr.*, 1903, 50, p. 849.

[2] *Quart. Jour. Med.*, 1909, 2, p. 289.

[3] *Arch. Int. Med.*, 1909, 4, p. 409.

[4] *Jour. Infect. Dis.*, 1909, 6, p. 245.

inulin moderately in 48 hours. Subcultures were now made on agar, in normal blood, and in the patient's blood, and transferred in each medium every second day for 30 days. At the end of this time the agar-grown culture fermented inulin in 24 hours. The subculture in blood failed entirely to produce acid, altho an abundant growth was obtained; it was then transferred to blood-agar for five days, then to sugar-free dextrose broth for 24 hours, and it now fermented inulin again, but more slowly than the agar-grown brand. Four weeks later all these strains after preservation on blood-agar fermented inulin slowly. The strain which failed to ferment after it was cultivated in the serum of the patient for one month fermented inulin after passage through a rabbit (see Table 1). The cultures isolated after death from the vegetations all fermented inulin slowly while those from the blood after death behaved the same as those isolated during life. It would seem as if the strains which did not ferment inulin had existed for some time in the blood of the patient, being probably descended from cocci washed into the circulation from the vegetations. Hence the inability to ferment inulin in a strain isolated from the blood in a single instance is not an argument against its being a pneumococcus.

Table 1 gives further observations with other strains of endocarditis cocci, with streptococci, Micrococcus rheumaticus, and pneumococci isolated from the blood of pneumonia. A glance at the table shows that the clotting of milk, the fermentation of saccharose, lactose, raffinose, salicin, and mannit, and growth or not on gelatin at 20° C. are of little value in differentiating streptococci from pneumococci. The length of time pneumococci are cultivated on artificial media determines largely whether they grow in gelatin or not. When first isolated they usually fail to grow. The fluctuation in the power to ferment inulin is very interesting. The endocarditic cocci usually ferment inulin slowly when first isolated; a short time later this power may be increased, only to disappear entirely after prolonged cultivation on blood-agar. The pneumococci from pneumonia at first ferment inulin strongly, but also lose it on long cultivation. If virulence is restored by animal inoculation the fermentative power returns. Streptococcus pyogenes and Streptococcus viridans, or "Streptococcus salivarius" of Horder, do not ferment inulin.

TABLE 1.

BIOCHEMICAL REACTIONS OF VARIOUS STRAINS OF PNEUMOCOCCI AND STREPTOCOCCI.

Strains of Pneumococci and Streptococci	Time Grown on Artificial Media	Milk Clotted	Acid in Saccharose	Acid in Lactose	Acid in Raffinose	Acid in Inulin	Acid in Salicin	Acid in Mannit	Growth in Gelatin at 20° C.	Character of Growth and Morphology in Broth	Green on Blood-Agar Plates
Pneumococci from endocarditis:											
Case 362. Strain isolated from first blood culture	2 days	±	+	+	+	+	0	−	0	Clumps in bottom; char. long chains	+
	3 months	+	+	+	+	0	+	0	+	Diffuse turbidity; diplococci	+
Case 362. Strain isolated in fifty-second blood culture	2 days	+	+	+	+	+	+	−	0	Long chains; clumps in bottom	+
Case 362. Strain from fifty-second blood culture after 5 rabbit passages	5 days	+	+	+	+	+	−	−	0	Diffuse turbidity; diplococci	++
Case 292	1 week	+	..	..	..	+	..	..	0	Long chains	+
Case 292	2 years	+	+	+	0	±	+	+	+	Diffuse turbidity	++
Case 293	5 days	±	..	..	..	+	..	..	0	Long chains	Slight
Case 293	2 years	±	+	0	0	0	+	+	+	Diffuse turbidity	+
Case M	2 weeks	+	−	−	−	+	−	−	+	Long chains	..
Case M	3 years	±	−	+	−	0	+	+	+	Diffuse turbidity	+
Case 409	1 week	+	++	+	0	+	+	−	+	Turbidity and clumps at bottom; short chains	+
Case 408	2 weeks	+	+	+	..	+	−	−	0	Diffuse turbidity; diplococci	+
Case 404	2 months	+	+	+	0	+	+	−	0	Clumps; no turbidity; long chains	+
Pneumococci from pneumonia:											
Case R	2 weeks	+	−	−	−	++	−	−	0	Diplococci	++
Case R	7 years	−	−	−	−	0	−	−	+	Diffuse turbidity; long chains	+
Case R2 Passages in rabbit	6 months	+	+	−	−	+	−	−	+	Diffuse turbidity; long chains	+
Case R3 Passages in rabbit	3 weeks	−	−	−	+	+	−	−	0	Long chains; capsules+	++
Case 245	Immed.	±	..	..	..	+	..	..	0	Diffuse turbidity; diplococci	++
Case 245	5 years	++	++	++	+	0	+	+	+	Diffuse turbidity; long chains	+
Case 245 After 4 passages in rabbit	2 weeks	+	+	+	+	+	−	+	0	Diffuse turbidity; diplococci; capsules	++
Case 210	Immed.	+	−	−	−	++	−	−	0	"	++
Case 210	5 years	+	+	+	+	0	+	+	+	Diffuse turbidity; long chains	+
Case 210 After 6 passages in rabbit	2 weeks	+	+	−	+	+	−	+	0	Diffuse turbidity; diplococci; capsules	++
Streptococcus viridans	6 months	+	+	+	+	0	0	0	+	Clumps; no turbidity; long chains	+
Streptococcus pyogenes	2 months	+	+	+	0	0	+	0	+	Diffuse turbidity; long chains	0 Hemolysis
Micrococcus rheumaticus	2½ years	0	+	+	+	±	+	−	+	Diffuse turbidity; some chains	+

When first isolated the endocarditis pneumococci form long chains and clumps in broth, often growing in tenacious masses at the bottom of the tube and producing no turbidity. Sooner or later after cultivation they grow in diplococcus forms with occasional short chains, while still later they grow again in longer chains but now produce a diffuse turbidity. Marked involution forms are common as in the case of pneumoccoci from pneumonia which have been cultivated for a long time (see Fig. 1).

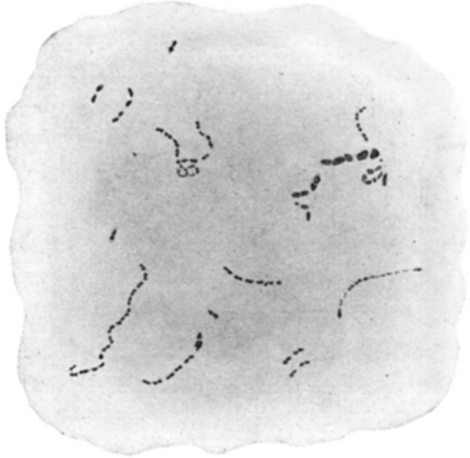

Fig. 1.—Smear from a 24-hour culture on blood-agar of pneumococcus cultivated on artificial media (chiefly on blood-agar) for seven years. Note long chains and involution forms. ×1,000.

The production of green by the endocarditis cocci on blood-agar when first isolated is always less than in those from pneumonia. In a number it was very slight when first isolated. Hemolysis about the colonies was never observed when fresh human blood was used in preparing the plates. After cultivation, as other characteristics of typical pneumoccoci return, the production of green is greater.

In this connection another point of importance must be mentioned. As stated, the effect of normal serum on these organisms is pronounced. Subcultures on blood-agar of cocci treated with serum repeatedly showed a greater production of green by those treated in normal serum than by those treated or "sensitized" in the serum of the patient. There was also marked difference in the growth of these organisms in normal serum and in patient's serum, and the injection of normal serum into the patient seemed to modify the organisms so that the production of green about the colonies on blood-agar became greater, chain formation less pronounced, and adherence to the surface of the agar less marked than before. In cases not injected with normal serum these characteristics seem to become more and more marked as the disease progresses. In a patient (Case 362, p. 429) who received many intravenous injections of normal serum the gradual diminution of the tendency of the organisms to adhere to surfaces was associated with an unmistakable diminution in the agglutinating power of the patient's serum. The limit of agglutination before the injections were begun was 1:3,000. This gradually came down until after 18 injections of normal serum it was 1:150. The subcutaneous injections of smaller doses in Case 408 seemed to have a similar effect, altho not so great. Whether diminution in agglutination was due to a lessened agglutinability of the bacteria or disappearance of agglutinins from the blood was not decided.

If the organisms in question are modified pneumococci, then we would expect that as the cultural characteristics of typical pneumococci return, the original pathogenic properties should also return. I have shown elsewhere[1] that the adherence to the surface and growth in clumps are closely related to the ability of these bacteria to produce endocarditis in animals. The cocci from the animals that died with endocarditis showed the original "special" characteristics, while in animals which died soon after inoculation from pneumococcemia or from pneumonia, the cocci in general gave the characteristics of typical pneumococci. Exactly similar results, but on a larger scale, were obtained in the animals inoculated with the bacteria from the present series. The production of pneumonia in animals with cocci from cases of endocarditis and the isolation after death of typical pneumococci from the blood and from the consolidated lung would seem to furnish crucial evidence that the endocarditis cocci are pneumococci. By means of plate cultures I was able to assure myself that the bacteria injected consisted of so-called modified pneumococci. Further, if the "special" characteristics of the modified pneumococci are the result of long growth in the circulating blood, as seems probable, then the prolonged cultivation of typical pneumococci in endocarditis serum in the test tube might modify them in a similar manner. Three strains of typical pneumococci from the blood of patients with lobar pneumonia, grown in the filtered serum from Cases 408 and 409 for a period of three weeks, assumed long chains with involution forms much as the organisms from endocarditis; they also became more agglutinable and produced in broth a sediment as well as a diffuse turbidity; fermentation of inulin was less marked, and the production of green on blood-agar diminished.

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[1] *Jour. Infect. Dis.*, 1909 6, p. 245.

AGGLUTINATION TESTS.

As cases of chronic infectious endocarditis with joint involvement frequently resemble cases of rheumatic fever, agglutination tests were made in order to determine whether agglutination might be of any diagnostic value (Table 2). The results may be summarized as follows: The strains from my cases of chronic pneumococcus endocarditis were all agglutinated by the serum in each case. None of these sera agglutinated Micrococcus rheumaticus (Poynton and Payne) or Streptococcus. The serum of the case of acute pneumococcic endocarditis did not agglutinate Streptococcus or Micrococcus rheumaticus, but it agglutinated the strains of pneumococci from chronic endocarditis (as well as its own strain). The rheumatism serum agglutinated strain 413 (from chronic endocarditis) to the same extent as normal serum; it did not agglutinate the other pneumococcic strains, but agglutinated distinctly Micrococcus rheumaticus

TABLE 2.

AGGLUTINATION OF PNEUMOCOCCI, STREPTOCOCCUS PYOGENES, AND MICROCOCCUS RHEUMATICUS BY SERA FROM CASES OF ENDOCARDITIS AND RHEUMATISM.

BACTERIA	SERA											
	Chr. Endoc. (408)		Chr. Endoc. (409)		Chr. Endoc. (413)		Ac. Pneu. Endoc. (412)		Ac. Rheum. (411)		Normal	
	1:20	1:1000	1:20	1:1000	1:20	1:1000	1:20	1:1000	1:20	1:1000	1:20	1:1000
Pneumococcus, chr. endoc. 293 (2 years)	+	0	++	0	++	0	–	0	0	0	0	0
Pneumococcus, chr. endoc. 209 (10 days)	+	0	+++	+	+++	0	++	0	0	0	0	0
Pneumococcus, chr. endoc. 413 (3 days)	++	–	++	–	++	+	–	+	+	–	+	–
Pneumococcus, chr. endoc. 362 (3 months)	+	0	+	0	+	0	–	0	0	0	0	0
Pneumococcus, chr. endoc. 292 (2 years)	++	0	+	0	+	0	–	0	0	0	0	0
Pneumococcus, ac. endoc. 412 (3 days)	+	0	+	0	++	0	+	0	0	0	0	0
Micrococcus rheumaticus (2½ years)	0	0	0	0	0	0	–	0	±	0	0	0
Pneumococcus pneumonia 410 (5 days)	0	0	+	0	++	0	0	0	0	0	0	0
Strept. pyog. (4 months)	0	0	0	0	–	–	0	0	+	0	0	0

The figures in parentheses in column headed by "Bacteria" give the time since the strains were isolated.

The bacteria used in these tests were grown in sugar-free dextrose broth for 24 hours and then heated at 60° C. for 30 m. The results were recorded after the mixtures had remained at 37° C. for 24 hours.

and Streptococcus. It would seem then that in chronic infectious endocarditis agglutination tests may give results of some diagnostic value. My results strengthen the observations by Meakins,[1] Tunnicliff,[2] and others to the effect that rheumatism may be a streptococcus disease.

ANIMAL EXPERIMENTS.

I have shown that the production of endocarditis in animals by pneumococci without injuring the valves is closely dependent on special characteristics of the cocci and on the largeness of the dose. It was noted that while the first injection rarely caused lesions or death it rendered the animal more susceptible to subsequent inoculations, and that animal passage had a marked effect on the morphology and other characteristics of the organisms. Horder[3] produced endocarditis in rabbits by repeated injections of huge doses of non-virulent or "saprophytic streptococci" as he calls them. Others have had similar results. The possibility of producing endocarditis without trauma of the valves under proper conditions seems settled, but a closer study of the changes in morphology and pathogenicity of the bacteria inoculated seems desirable and the animal experiments given in Tables 3, 4, and 6 were undertaken for this purpose.

The development of only very few cases of endocarditis in this series as compared with the former is explainable because the number of bacteria inoculated was too small or the special characteristics were largely lost at the time of injection either by prolonged cultivation or as the result of the injection of normal serum into the patient.

Table 3 gives the results obtained from the injection of a single dose. They show a marked increase in "virulence" of the bacteria when cultivated in the patient's blood as compared with those grown on artificial media, quite independently of the place of inoculation. A single injection in all but one instance of even a few bacteria proved fatal (182, 187, 191, 194, 197, and 50). This virulence is also observed in animals which had recovered from a previous inoculation at the time of the second injection (Table 4). Continuous cultivation in fresh normal blood has a similar, but not so marked an effect (203, Table 3; 183, 52, Table 4). The primary injection of the bacteria

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[1] *Can. Jour. Med. and Surg.*, 1909, 25, p. 71.

[2] *Jour. Infect. Dis.*, 1909, 6 p. 346.

[3] *Quart Jour. Med.*, 1909, 2' p. 289.

TABLE 3

SUMMARY OF EXPERIMENTS ON RABBITS WITH SINGLE INJECTION OF PNEUMOCOCCI FROM ENDOCARDITIS.

No.	Inoculations: Date; Dose; Place	Time Since Organism Was First Isolated	Date and Cause of Death	Remarks and Postmortem Findings
182	1/8, Pn. 362.—4 blood-agar slants washed off in 3 c.c. of 24-hr. culture in patient's blood; intraperitoneally	4 days	1/9 Pneumococcemia	Blood crowded with typical lanceolate pneumococci. Subcultures show normal type pneumococci
187	1/28, Pn. 362^{15}*.—2 c.c. 362 blood after 48 hours' incubation intraperitoneally. No organisms seen in smears but cultures on blood-agar yield 10 colonies per c.c. Subcultures from all colonies yield modified pneumococci	2 days	1/12 Pneumococcemia	No anatomic lesions. Blood cultures on blood-agar yield moderate number of greenish colonies of typically lanceolate encapsulated diplococci, which are quite freely susceptible to phagocytosis by human leukocytes in human serum (see animal 188, Table 4)
191	2/17, Pn. 362^{41}.—24-hr. culture in 1 c.c. 362 blood (abundant growth) intraperitoneally. All subcultures from 10 colonies on blood-agar show modified pneumococci, but they no longer adhere to surface	2 days	2/25 Pneumococcemia	No gross lesions. Smears of bone marrow show many diplococci. Blood and peritoneal smears sterile. Blood cultures yield few green colonies of normal type of pneumococcus, susceptible to phagocytosis and not highly virulent (see animal 193, Table 4)
194	3/1, Pn. 362^{57}.—2 c.c. 362 blood in which pneumococcus was grown 48 hours intraperitoneally	3 days	3/27 Chloroformed	Marked emaciation; hemoglobin 55 per cent; no gross lesions; culture sterile
192*b*	2/27, Pn. 362^{51}.—2 c.c. 362 blood intraperitoneally (2 c.c. plated out at same time yield three colonies pneumococci all of modified type, but no longer adherent to surface)	½ day	4/11 Pneumonia	Marked loss in weight; pneumonia, acute tracheo-bronchial lymphadenitis. Pneumococci isolated from heart's blood and consolidated lung have characteristics of ordinary pneumococci. Moderately virulent altho quite susceptible to phagocytosis (see 219 below, and 185, Table 4)
189	2/17, Pn. 362^{41}.—5 c.c. 24-hr. blood broth culture intravenously, 5 c.c. intraperitoneally, and 5 c.c. subcutaneously	2 days	3/18 Pneumococcemia	No gross lesions. Cocci isolated of normal type
190	2/17, Pn. 362^{41}.—Same as 189 +5 c.c. normal human defibrinated blood intraperitoneally	2 days		Remained well
50	8/6, Pn. 408.—4 c.c. intravenously of 48-hr. ascites broth culture after cultivation in 408 blood for four weeks	4 weeks	8/26 Endocarditis	Endocarditis of tricuspid valve. Anemic infarct left lower lobe. Strain isolated resembles one injected
219	4/4, Pn. 192*b*.—2 c.c. 24 hr. broth culture and one blood-agar slant intraperitoneally	11 days	4/22 Pneumococcemia	No gross lesions. Organism isolated from heart's blood in large numbers and of normal type
203	3/24, Pn. 311^{8}.—After cultivation in normal human serum for three weeks; 5 c.c. 24-hr. milk culture and two ascites agar slants intraperitoneally	2 years	4/21 Infection	Marked emaciation. No peritonitis; no endocarditis but abscess of liver. Pus yields pure culture typical pneumococcus. Inoculation of agar-grown culture in another animal caused no lesions

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* The figures to the right and above the number of the strain of pneumococcus indicate the blood culture in which the strain injected was isolated, i.e., 362^{15} indicates the strain isolated in the fifteenth blood culture in Case 362.

TABLE 3.—*Continued.*

No.	Inoculations: Date; Dose; Place	Time Since Organism Was First Isolated	Date and Cause of Death	Remarks on Postmortem Findings
55	8/14, Pn. 408.—5 c.c. intravenously ascites broth culture of organism isolated from blood of 49, day after injection	5 weeks	9/7 Paralysis	Ill for several days. Then recovered. Died from ascending paralysis which came on four days before death. No anatomical lesions. No meningitis. Blood cultures negative except from spinal cord which yields a pneumococcus
195	3/6, Pn. 362^{57}.—10 c.c. 48-hr. broth culture into left ventricle	7 days	4/12 Pericarditis	Marked emaciation; huge adhesive and fibrinous pericarditis. Organism isolated from heart's blood and pericardium shows modified characteristic, but not adherent to surface of agar
196	3/3, Pn. 362^{57}.—10 c.c. broth culture into left ventricle	7 days	3/13 Endocarditis and pericarditis	Fibrinous pericarditis; beginning endocarditis. Organism isolated in pure culture grows in clumps moderately adherent to surface
197	3/6, Pn. 362^{57}.—7 c.c. 24-hr. broth culture into right and left ventricle	7 days	4/1 Endocarditis and pericarditis	Endocarditis of aortic valve; mural endocarditis of left ventricle. Organism isolated resembles original culture closely. Adhesive and fibrinous pericarditis
201	3/22, Pn. 362^{8}.—Three blood-agar slants and three ascites agar slants intraperitoneally and into heart	63 days	4/20 Chloroformed	Had lost some in weight. Adhesive and fibrinous pericarditis. No other gross lesions. Heart's blood sterile
192	2/17, Pn. 362^{41}.—10 c.c. 24-hr. broth culture and one blood-agar slant intraperitoneally	2 days	3/15 Thrombosis of portal vein	No endocarditis but thrombosis of terminal radicles of portal vein. Smears from thrombus rich in diplococci. Cultures show modified pneumococci
205	3/24, Pn. 362^{8}.—10 c.c. ascites broth culture into heart and 10 c.c. intraperitoneally	65 days	4/19 Pericarditis	Fibrino- and fibrous pericarditis No other lesions. No endocarditis. No peritonitis. Cultures from heart's blood sterile. Smears from pericardium show modified pneumococcus but it does not adhere to surface of agar

isolated before the injections of normal serum in Patient 362 were begun, and cultivated on artificial media, never proved fatal either intravenously or intraperitoneally or both. After continuous cultivation in the patient's blood for four weeks (50) a single intravenous injection caused death from endocarditis. Injection of the bacteria, cultivated on artificial media for a considerable time, directly into the heart always produced pericarditis and usually also endocarditis. No increase in virulence could be detected in pneumococcus 362 after it showed definite reversion to the normal type, the result apparently

TABLE 4.

REPEATED INJECTIONS OF RABBITS WITH STRAINS OF PNEUMOCOCCUS FROM ENDOCARDITIS.

No.	Inoculations: Date; Dose; Place	Time Since Organism Was First Isolated	Date and Cause of Death	Remarks and Postmortem Findings
181	12/16, Pn. 362.—10 c.c. broth culture and one blood-agar slant intravenously; 10 c.c. intraperitoneally and subcutaneously	10 days	1/9 Pneumococcemia	Prompt recovery from first injection. Death in 36 hrs. after second injection. Pneumococci very numerous in blood, lanceolate, encapsulated, and subcultures give normal type pneumococci
	1/7.—Four blood-agar slants washed off in 3 c.c. of 24-hr. culture in patient's blood intraperitoneally	4 days		
183	1/11, Pn. 362^4.—6 c.c. 48-hr. culture in normal blood (growth abundant) intraperitoneally	7 days	3/4 Inanition; thrombosis of coronary veins	Ill for one or two days after first injection, then recovery. Very ill for three days after second injection, then gradually improved for a time, but later lost markedly in weight. Thrombosis right coronary vein. Organism isolated in pure culture from thrombus; heart's blood sterile; modified type of pneumococcus
	1/28, Pn. 362^{11}.—Three blood-agar slants intraperitoneally	2 days		
184	1/11, Pn. 362.—Five blood-agar slants washed off in broth intraperitoneally	27 days	2/22 Unknown	Seemed ill for day or two, then recovered both after first and second injection. Cultures from heart's blood and pericardial fluid sterile. No lesion.
	1/28, Pn. 362^{15}.—Three blood-agar slants +2 c.c. normal human blood intraperitoneally	2 days		
185	1/28, Pn. 362^{15}.—Three blood-agar slants intraperitoneally	2 days	5/5 Pneumonia	Ill for day then complete recovery. Recovered more promptly than 184 after second injection, nor was illness as great (see 192, Table 10)
	2/17, Pn. 362^{41}.—10 c.c. ascites broth + one blood-agar slant + 2 c.c. normal human blood intraperitoneally	37 days		
	192*b*.—3 c.c. 24-hr. broth culture and one blood-agar slant intravenously	10 days		Apparent recovery for six days after third injection, then began to breathe rapidly, etc. Pneumonia, acute peribronchial lymphadenitis. Cultures from blood sterile; from lung encapsulated diplococci of normal pneumococcus type
188	2/14, Pn. 362^{15}.—Two agar slants of organism isolated from animal 187, Table 10, intraperitoneally	2 days	4/22	Prompt recovery after severe illness for a day. Severe illness following second injection but finally recovered. Seemed perfectly well when injected third time. Severe illness due to large dose directly into heart. No hypersensitiveness. Death from pneumococcemia in two days after third injection. Healing serofibrinous pericarditis. Blood cultures yield diplococci with short chains.
	3/24, Pn. 362^1.—10 c.c. 48-hr. ascites broth culture into heart	65 days		
	4/21.—10 c.c. intraperitoneally. 3 c.c. broth culture Pn. from rabbit 192, and surface growth of blood-agar slant, intravenously	10 days		
193	3/1, Pn. 362^{41}.—After passage through animal 191, three blood-agar slants intraperitoneally	4 days	3/27 Chloroformed	Very ill after first injection but prompt recovery after second injection. Blood cultures after death sterile
	3/6, Pn. 362^{57}.—3 c.c. of 48-hr. blood-broth culture intraperitoneally	7 days		
49	8/6, Pn. 408.—5 c.c. ascites broth culture after cultivation in 408 blood for four weeks, intravenously	4 weeks	9/7 Chloroformed	Day after injection 250 pneumococci per c.c. of blood. Very ill for short time but recovered completely. No lesions. Blood culture sterile
	8/14, Pn. 408.—The same as above after cultivation on blood-agar for five days	5 weeks		

TABLE 4.—*Continued.*

No.	Inoculations: Date; Dose; Place	Time Since Organism Was First Isolated	Date and Cause of Death	Remarks and Postmortem Findings
48........	8/3, Pn. 408.—6 c.c. ascites broth culture intravenously 8/14, Pn. 408.—5 c.c. ascites broth after cultivation in 408 blood for four weeks and on blood-agar five days, intravenously	4 weeks 5 weeks	9/7 Chloroformed	Not as ill as 44, Table 10, which had received Pn. 409 three weeks previously; 250 pneumococci per c.c. of blood. Control dead fourth day. Complete recovery. Very ill for two days then improved five days after injection. Arthritis of left front ankle joint. Recovered. Autopsy: No lesions; blood sterile.
52........	8/6, Pn. 408.—4 c.c. ascites broth culture after growth in normal blood for 4 weeks, intravenously 8/14, Pn. 408.—5 c.c. ascites broth culture after cultivation in normal blood for 4 weeks, and on blood-agar 5 days, intravenously	4 weeks 5 weeks	8/16 Pneumonia	Recovered promptly after first injection. Very ill after second. Death two days after injection from beginning pneumonia. Cultures from heart's blood and lung yield typical pneumococci

of numerous intravenous injections of normal human serum in the patient.

Normal serum being found to raise the phagocytic and destructive power of the patient's blood to the normal point when low, it is interesting to note that the subcutaneous injection of normal serum into the infected rabbit served to protect the animal from otherwise fatal doses. This was found to be true for the first as well as subsequent inoculations (184, 185, Table 4; 190, Table 3).

The aforementioned increased susceptibility of animals to subsequent injections of these organisms is confirmed by the results obtained in the present study (Table 4). The hypersensitiveness is roughly proportionate to the dose, but quite independent of the place of inoculation, i.e., intraperitoneal injections sensitize to subsequent intravenous injections and vice versa. In order to produce death promptly after a second injection relatively large doses are necessary. The seemingly increased susceptibility to infection might in reality be an expression of an active immunity and death the result of an overwhelming intoxication from rapid destruction of the organisms injected. In regard to this point there is this to say, that the "sensitized" animals present none of the usual symptoms of anaphylaxis following the second injections—the reaction is different from the anaphylactic phenomenon following the injection of foreign proteids. Moreover, a study of peritoneal smears during life, instead of showing a more rapid phagocytosis and destruction of cocci after the second

TABLE 5.

SENSITIZATION OF RABBITS WITH PNEUMOCOCCI FROM ENDOCARDITIS.

No.	Inoculations: Date; Dose; Place	Time Since Organism Was First Isolated	Date and Cause of Death	Remarks and Postmortem Findings
44........	7/9, Pn. 409.—Washed and suspended in NaCl after growth in 3 c.c. 409 serum for 48 hours; 2 c.c. intravenously; 1 c.c. intraperitoneally 8/3, Pn. 408.—6 c.c. ascites broth culture intravenously (see 48, Table 4, the control)	4 days 4 weeks	8/5 Pneumonia	Ill for only day or two after injection of Pn. 409; complete recovery. Day after second injection, animal showed dyspnea and 5,600 pneumococci per c.c. of blood. Lobar pneumonia, stage of red hepatization; beginning pericarditis; no endocarditis; no arthritis. Typical pneumococci from heart's blood and consolidated lung (see plate)
43........	7/9, Pn. 409.—2 c.c. intravenously and 1 c.c. intraperitoneally of 409 serum in which Pn. 409 was grown for 48 hrs. (see 44) 8/6, Pn. 408.—5 c.c. ascites broth culture after cultivation in 408 blood for four weeks, intravenously (see 49, Table 4) 8/14, Pn. 408.—8 c.c. ascites broth culture after cultivation in 408 blood for four weeks and then on blood-agar five days, intravenously	4 days 4 weeks 5 weeks	9/7 Chloroformed	After first injection seemed ill for two days, then recovered. Blood cultures two days later sterile. Seemed very ill day after second injection. Breathes rapidly; 13,000 whites; 180 colonies pn. per c.c. of blood. Rapid breathing kept up for five days longer, then recovered. Recovery after third injection prompt. Blood cultures day after sterile. Autopsy: No lesions; blood cultures sterile
4........	7/16.—3 c.c. broth culture intravenously of pneumococcus isolated from pneumonia in man 8/8, Pn. 409.—6 c.c. ascites broth culture intravenously	1 week 2 months	9/7 Chloroformed	Prompt recovery after first injection. Ill for a time after second injection. No evidence of hypersusceptibility. Autopsy: Healed adhesive pericarditis; otherwise normal; blood sterile
45........	7/7, Pn. 412.—(From acute pneumococcus endocarditis) 6 c.c. ascites broth culture+growth on 3 blood-agar slants, intraperitoneally 8/9, Pn. 409.—6 c.c. ascites broth culture intravenously	7 days 3 months	9/7 Chloroformed	Ill for short time, then complete recovery. First injection did not render animal susceptible to second injection. Recovery complete. No loss in weight. Autopsy: No lesions; blood sterile
47........	7/7, Pn. 410.—8 c.c. ascites broth culture from pneumonia intravenously 8/9.—6 c.c. intraperitoneally of ascites broth of Pn. 412 from case of acute pneumococcus endocarditis	3 weeks 2 months	9/7 Chloroformed	Ill for a time, then complete recovery No evidence of increased susceptibility. Prompt recovery. Autopsy: No lesion; culture sterile

or third injection, show an actual diminution in phagocytosis and a slower destruction than after the first injection. Again, cultures show actual multiplication of organisms in both the animals dying promptly from pneumococcemia without localization as well as in those which died later from endocarditis and pneumonia after a second injection. These facts would seem to prove that death must be due to increased susceptibility to infection.

It is a well-known fact that injection of pneumococci from pneumonia and other sources may produce an increased resistance to subsequent injections. Prolonged cultivation on artificial media, a short residence in normal serum, and animal passage, as already pointed out, have a pronounced effect on the morphology and cultural characteristics of these organisms. They become more like pneumococci from pneumonia. See Figs. 2, 3, 4, 5. Table 4 shows that prolonged cultivation on artificial media and cultivation in normal serum reduce the sensitizing power of the organisms to a definite degree, while animal passage takes away nearly all this property (184, 188, 193, 49, 48). In other words, reversion in morphology to the normal type occurs with the return of original pathogenic properties. In this connection there is still another point of interest. If these strains of pneumococci coming from different cases of endocarditis, and showing degrees of modification, belong to the same group, then we should expect that the injection of one strain would render the animal hypersensitive to the other strains also. In Table 5 it is shown that this is true. Moreover, the increased susceptibility is found to extend to moderately virulent pneumococci from pneumonia and from acute pneumococcus endocarditis. This fact is in accord with the idea that the strains studied are modified pneumococci.

Against the idea that in the animals dead from pneumococcemia or pneumonia in which the cocci isolated after death resembled typical pneumococci I was dealing with a mixt culture, it must be stated that this point was carefully controlled by plating out part of the material at the time of inoculation and studying the organisms from the individual colonies (187, 191, 192*b*, Table 3). In each instance only modified pneumococci were found. Similar observations were made of the colonies obtained on the blood-agar plates from the blood cultures obtained from the patients. In no instance were typical pneumococci isolated. Furthermore, the postmortem findings in Cases 362 and 408 (pp. 429, 431) showed no lesions which would lead one to suspect pneumococci of the normal type in the circulating blood

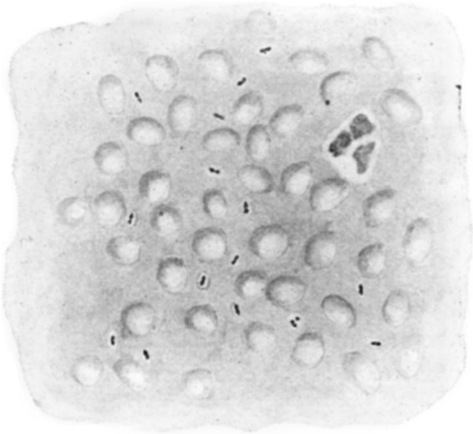

FIG. 2.—Typical encapsulated diplococci in smears of blood of rabbit dead from injection with modified pneumococcus of endocarditis. ×1,000.

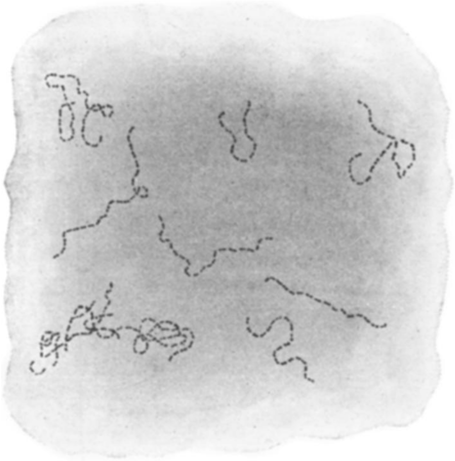

FIG. 3.—Pneumococcus 409 grown in serum 409 for 10 days. Note the small size of the cocci and the long chains. The cocci in the chains appear as diplococci. ×1,000.

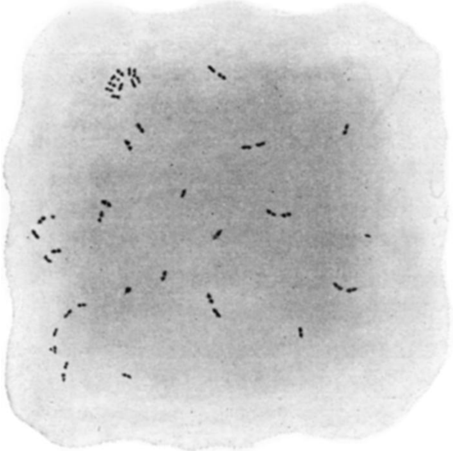

FIG. 4.—The same strain as Fig. 3 cultivated on blood-agar for 48 hours and in normal blood for three days, transfers being made daily. Note absence of chains and the larger size of the cocci which are often lanceolate in shape. ×1,000.

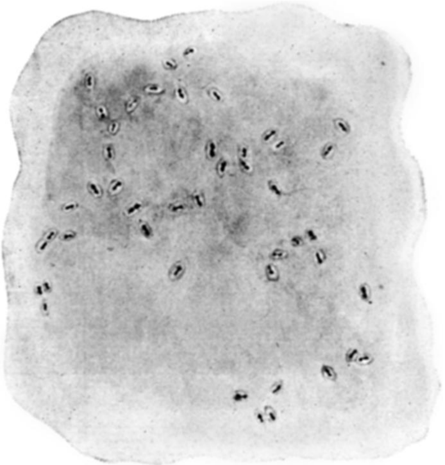

FIG. 5.—Buerger's capsular stain of pneumococci in rabbit blood after death from inoculation with pnuemococcus in Fig. 1 treated with extract of virulent pneumococci. ×1,000.

at the time the animal experiments were made. Spontaneous infection from outside sources is inconsistent with the results obtained in the animals which died of pneumococcus pneumonia. The animals were caged separately and no epidemic occurred among the other animals at the time of the experiments.

A study of the peritoneal smears in the animals injected intraperitoneally for the first time with cocci grown artificially showed marked phagocytosis and an exceedingly rapid destruction of the bacteria, the animals recovering promptly. When injected the second or third time with the same cocci or the first time with those grown in the patient's blood, both phagocytosis and destruction, while much delayed, were still pronounced, and the animal either succumbs in a short time from pneumococcemia or pneumonia, or later from endocarditis or pericarditis.

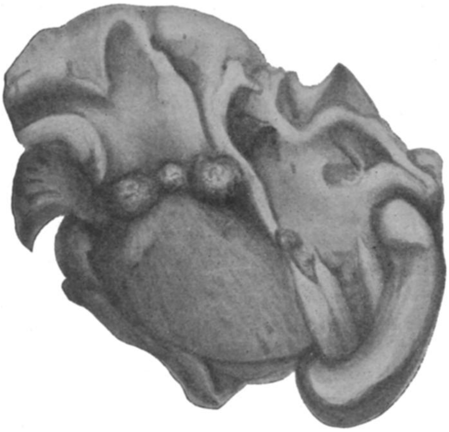

FIG. 6.—Experimental endocarditis in rabbit. Normal size. Three weeks before death the rabbit was given an intravenous and intraperitoneal injection of pneumococcus 408 after cultivation in the patient's serum for four weeks. The valves were not previously injured.

After death, smears from the blood, the pericardium, and the vegetations showed remarkably little phagocytosis; the organisms isolated from the blood or from the lesions, while quite susceptible to phagocytosis by human leukocytes in human serum, were not so freely taken up as previously and showed a corresponding increase in virulence, which was promptly lost on artificial cultivation. The strains isolated from those which died from endocarditis or pericarditis were more freely taken up than those dead from pneumococcemia. No difference could be established in this respect between those isolated from the animals dead from a primary or subsequent injection.

The morphology of the cocci isolated after death on the one hand, and the character of lesions produced on the other, show a striking parallelism. Thus in 13 out of 20 instances in which this point was studied the pneumococci isolated resembled closely typical pneumococci (see Fig. 2). Seven of these died of pneumococcemia without localization and one of pneumonia, one of abscess of the liver, and one of an ascending paralysis (55, Table 3). This animal was given a single injection of organism 408 after one animal passage. Of the remaining seven in which death occurred later, the organisms resembled more or less closely the ones injected; five showed endocarditis or pericarditis, or both; two thrombosis, one of the coronary artery and the other of the portal vein.

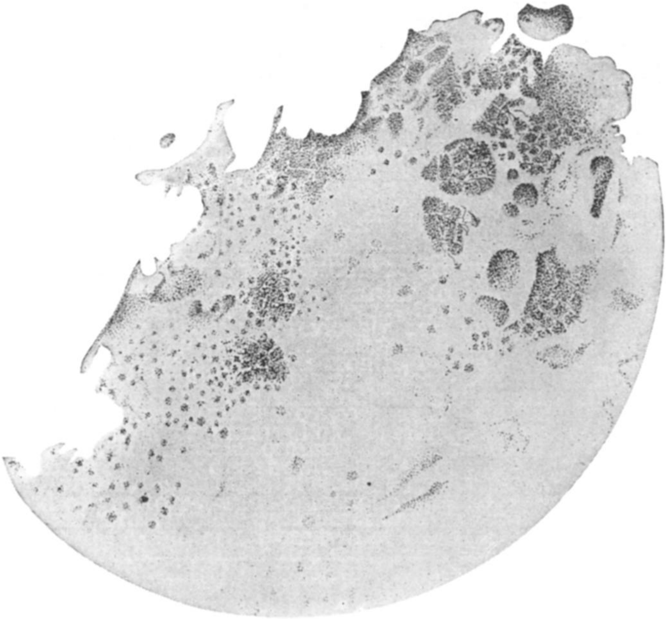

FIG. 7.—Gram strain of a section of vegetation from experimental penumococcus endocarditis in rabbit. The dark areas are collections of pneumococci imbedded in a fibrinous network. Their number and size increase as the free surface is approached. ×100.

The specific affinity of these organisms for vascular endothelium, endocardium and pericardium especially, is striking. Peritonitis never developed, notwithstanding the fact that the injections were made intraperitoneally in most of the animals. Arthritis developed in only one animal (48, Table. 4), from which it recovered. Subcutaneous abscesses never developed at the site of inoculation. It is interesting to note that pneumonia developed late (10 to 40 days) in the two rabbits receiving only one dose and promptly (2 days) in the two in which it developed after the second injection. The consolidation was not always distributed in lobes but the line of demarkation was sharp and the microscopic sections show the cellular infiltration characteristic of fibrinous pneumonia.

SUMMARY.

The reasons, then, for looking on the cocci isolated from the cases studied as modified pneumococci may be briefly summarized as follows: (1) The chains are made up of distinct elongated diplococci, often lanceolate in shape, frequently showing involution forms similar to those of ordinary pneumococci cultivated on blood-agar for a long time. (2) They always produce a variable amount of green on blood-agar plates, but usually much less than the typical pneumococci, and never hemolysis. (3) All the strains ferment inulin. (4) Colonies when first isolated adhere more or less closely to the surface of blood-agar slants and grow in clumps at the bottom of test tubes of broth or other liquid media and in the fibrin clot of the blood cultures, leaving a clear fluid. Sooner or later after cultivation on artificial media these special characteristics are lost; they no longer form chains, but are typical diplococci instead; no longer adhere to the surface of agar, and grow with a diffuse turbidity in broth. The more marked these characteristics are in the beginning, the longer it takes for them to disappear. (5) Cultivation in normal serum or blood and animal inoculation may call forth promptly the usual pneumococcal characteristics, i.e., typical lanceolate diplococci, often encapsulated, which produce more green on blood-agar and otherwise grow exactly as pneumococci from pneumonia, often result, as observed also by Buerger and Ryttenberg.[1] Recultivation in the patient's serum brings back the "special" characteristics, which is true also of strains which have lost them by cultivation on artificial media. (6) The special characteristics show great variations in the different strains studied. The more chronic the course of the disease, the more marked they are, and vice versa. In Cases 362 and 408 they were pronounced, while in Case 409, which ran a more acute course, they were less marked. (7) Reversion to the normal type, morphologically and culturally, is associated with return of the pathogenic properties more or less characteristic of typical pneumococci. (8) The pneumococcal organisms are agglutinated by the sera from the endocarditis cases in high dilution; and the sera also agglutinate typical pneumococci, but not streptococci. (9) Cultivation of pneumococci from pneumonia in fresh serum from cases of chronic endocarditis produces changes similar to those observed in the cocci isolated from the blood of cases of chronic infectious endocarditis.

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[1] *Jour. Infect. Dis.*, 1907, 4, p. 600.